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Ameliorative effects of the homeopathic medicine Lycopodium 200c and extract of *Phyllanthus emblica* in cadmium-induced neurotoxicity in mice

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Cadmium is an extremely toxic heavy metal and causes neurotoxicity by inducing oxidative stress and membrane disturbances in brain. *Phyllanthus emblica* and Lycopodium 200c have anti-oxidative properties and are able to remove the cadmium-produced free radicals. This study investigates the role of Lycopodium 200c and *Phyllanthus emblica* (amlaki) in ameliorating the toxic effects of cadmium on the brain of mice. Swiss albino mice were used and divided into four different sets with one control, one induced, one with amlaki and other with both amlaki and Lycopodium treatment. To observe the changes, tests for brain acetylcholinesterase along with Mg^{2+} ATPase activities were performed. Results show that cadmium toxicity leads to decrease in enzymatic activities which can be reversed by the effects of amlaki and Lycopodium 200c.

Keywords: Antioxidative properties, cadmium, free radicals, oxidative stress, toxicity.

AMONG the well-known toxic heavy metals, cadmium is very common which can found in the environment in various compounds, e.g. oxides, sulphides, chlorides, etc. It enters the animal body through food and drinks and is transported through blood and gets easily accumulated in different organs including liver, kidney, testes, lung, etc. and causes severe toxicity. It also acts as a harmful neurotoxin in mammalian brain[1,2]. Cd-induced toxicity is responsible for the generation of reactive oxygen species (ROS)[3,4]. Cadmium also influences the activity of certain enzymes such as the uptake of catecholamines[5] affecting the levels of several neurotransmitters and also affects antioxidant status[6]. It blocks adrenergic and cholinergic synaptic transmissions[7]. There are many chelating agents which form a chelator–metal complex resulting in a decrease of tissue cadmium concentration. Acetylcholinesterase (AChE) is important in the Ach (acetylcholine) cycle[8]. Besides, it is co-released from the dopaminergic neurons[9]. Studies have been conducted to

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assess the chemo-preventive roles of several natural antioxidants in heavy metal induced toxicities[10,11]. They are considered effective in preventing oxidative stress-related abnormalities and as potential remedial agents in various diseases. They can restrain the decreased ATPase activities and increased Cd-induced oxidative stress[12]. As *Phyllanthus emblica* (amlaki) and *Lycopodium clavatum* contain many such antioxidants, they are considered effective in treating such kinds of metal toxicity. Along with their antioxidative properties, they also have antitumorigenic, anti-inflammatory and anti-toxic effects. The most useful form of *Lycopodium clavatum* is its alcoholic diluted form that is broadly used in homeopathy. For this study, the homoeopathic medicine Lycopodium 200c was used. We study the antagonistic role of antioxidants and flavonoids present in both amlaki and Lycopodium 200c on cadmium-induced neurotoxicity in mice. A short-term (40 days) *in vivo* study was conducted to measure AChE and Mg^{2+} ATPase activities of mice brain.

The animals were handled and kept under normal laboratory conditions maintaining all Animal Ethical Rules. The animals were provided with normal food and water. Swiss albino mice (~20 g each; 4 mice in each set) were used as experimental model and divided into four different sets such as (i) SI or normal control (NC) set: daily administered with normal food and water and without any treatment, (ii) SII or cadmium-induced (Cd) set: administered with cadmium chloride in addition to normal feeding, (iii) SIII or cadmium and amlaki treated (Cd + A) set: administered with both cadmium and amlaki along with normal food and water and (iv) SIV or cadmium, amlaki and lycopodium (Cd + A + L) treated set: administered with cadmium, amlaki and lycopodium along with normal food and water. Cadmium chloride was used as the source of cadmium and a high dose of 100 mg/kg body weight was given. Amlaki juice and Lycopodium 200c were administered at a dose of 10 µl/25 g body weight.

AChE activity was assessed from mice brain according to the method of Ellman *et al.*[13], where acetylthiocholine iodide was used as a substrate. The AChE, present in samples, hydrolyses acetylthiocholine iodide into acetate and thiocholine. In the next step, 5-thio-2-nitrobenzoic acid was formed when thiocholine reacts with 5,5′-dithiobis-2-nitrobenzoic acid. The developing yellow colour was then measured by spectrophotometric analysis at 412 nm. The brain Mg^{2+} ATPase activity was determined following the method of Ohinishi *et al.*[14]. The Mg^{2+} ATPase activity in 0.1 ml of tissue homogenates was determined by adding 0.1 ml of 125 mM *Tris*-HCl (pH 8), 0.1 ml 50 mM $MgCl_2$, 0.1 ml of 10 mM ATP. These final mixtures were incubated for 10 min at 37°C. Finally, the reaction was stopped by introducing 1 ml ice-cold 10% TCA and centrifuged at 1500 *g* for 10 min. The liberated inorganic phosphate (Pi) from the protein free test samples of Mg^{2+} ATPase activities was measured following the method of Eibl and Lands[15]. The developing colour was measured spectrophotometrically at 820 nm. The activities of these ATPase enzymes in tissue homogenates were expressed as µg Pi liberated per minute per mg of protein. Statistical analysis was carried out using the SPSS statistical package version 12.0. The results are expressed as mean ± SD and the data analysis was performed by one-way analysis of variance (ANOVA) followed by Turkey's multiple comparison tests when there is a significant *F* test ratio. The level of significance was fixed at $P = 0.05$.

The effect of cadmium on brain AChE activity of mice along with antagonistic effects of Lycopodium 200c and amlaki is presented in Figure 1. It shows that the activities of mice AChE were significantly decreased ($P < 0.05$) in cadmium-induced group when compared with normal control group. The activities are increased in experimental groups compared to cadmium-induced group. In experimental sets, the data are significant in case of dual treatment, i.e. with both Lycopodium 200c and amlaki ($P < 0.05$). Figure 2 represents the result of Mg^{2+} ATPase activities on the brain of mice. This figure shows that the activities of Mg^{2+} ATPase are decreased in cadmium-induced group compared to the normal control group. The activities of Mg^{2+} ATPase are significantly higher ($P < 0.05$) in other two groups, i.e. in Cd + A and Cd + A + L groups than in cadmium-induced group.

The enzyme AChE is effective in detecting the neurotoxic effects of certain heavy metals including cadmium. Studies have suggested that free radical production is partly associated with decreased activity of brain AChE[16] which leads to the accumulation of acetylcholine causing cholinergic hyperactivity, convulsion, status epilepticus, etc.[17]. Cadmium-related neurotoxicity also alters the neurotransmitter release mechanism and sometimes blocks the Ca^{2+} influx by following action potential through membrane channels into the nerve terminal. Cadmium is well known for its production of ROS. A significant reduction of intracellular thiols and antioxidants has been seen after the interaction of cadmium with mitochondrial sites[18]. It was also found that Cd acts as metal inactivator of the enzymes and induces a conformational change of the protein which leads to the formation of an 'unreactive' enzyme species[19]. Cadmium-induced free radical production in the brain of the mice interferes with the antioxidant defence system and leads to an alteration of the structural integrity of membrane lipids and membrane-bound enzymes, for e.g. different ATPases[20]. Figure 1 shows a decreased AChE activity due to cadmium induction. Figure 2 shows the decreased levels of membrane-bound ATPases in brain of cadmium intoxicated mice also conforms to the above explanation. Mg^{2+} ATPase maintains the high intracellular Mg^{2+} level in brain. Its changes can control the protein synthesis rate and cellular growth[21].

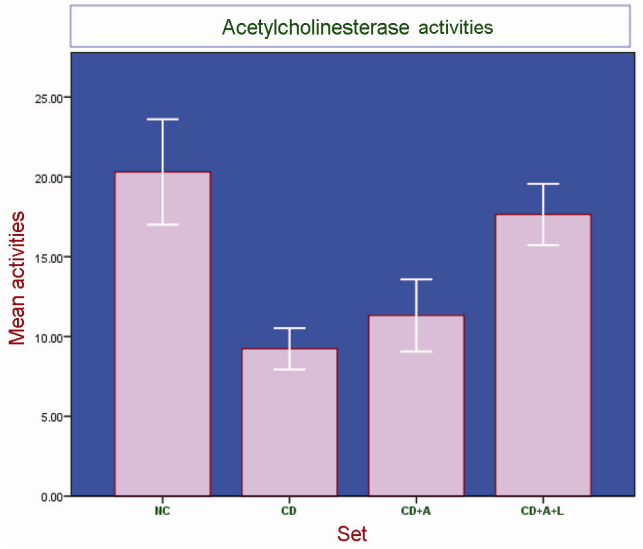

Figure 1. Acetylcholinesterase activities of mice from different sets. The bar diagram shows low AChE activities in cadmium-induced set than others.

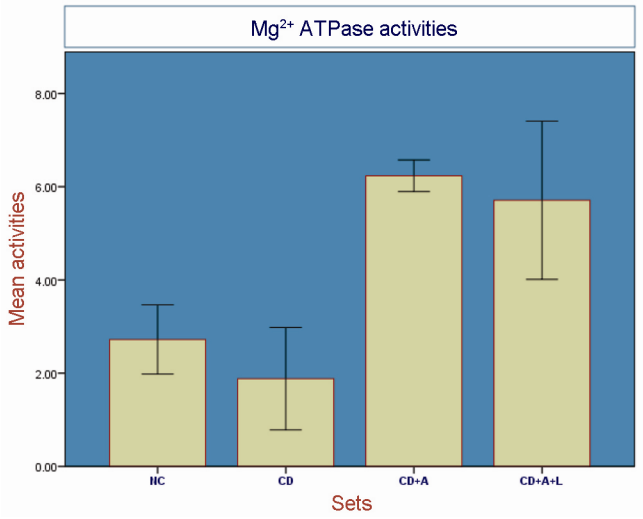

Figure 2. Bar diagram showing Mg^{2+} ATPase activities of mice from different experimental sets. Diagram depicts low activities in cadmium-induced set but increased activities in normal (NC) and treated sets.

According to various studies, it was seen that Cd acts as a potent inhibitor of brain Mg^{2+} ATPase and choline transports of synaptosomes. It interacts either by inhibiting or stimulating the adenylate cyclase activity, depending upon the cationic concentration by interacting with an enzymatic site closely related to the allosteric site of the regulatory unit of the Cd ATPase complex. The decreased activity of ATPase could also be due to the SH binding nature of Cd or through its oxidative stress in brain[22].

Flavonoids such as quercetin and kaempferol, the active compounds of Indian gooseberry, effectively impair with angiogenesis[23]. Besides, quercetin also proved that it could fight neurotoxic elements[24] and thus reduce the adverse effects of cadmium. Amlaki contains antioxidants such as emblicanin A and B and is a rich source of vitamin C, all of them together work against cadmium-induced toxicity[25,26]. Besides, the homeopathic medicine Lycopodium 200c contains many active compounds such as lycopodine, clavatine, epigenin, clavatoxine, ferulic acid, selagine, lycoflexine, lycofoline[27], etc. The combined effect of all these active compounds renders protection against such kind of toxic environment[28]. Their effects can be seen from the results. Figures 1 and 2 respectively, show the low AChE and Mg^{2+} ATPase activities, caused by cadmium which are reversed by treatment with amlaki juice and Lycopodium 200c. In Figure 1, it is seen that the combined effects of Lycopodium 200c and amlaki juice are more pronounced in reversing the situation than with amlaki juice alone. The treatment with amlaki alone (Figure 2) is more effective than the combined effects of Lycopodium 200c and amlaki juice.

From the data in presented in this study, it can be concluded that the *Phyllanthus emblica* and Lycopodium 200c have potential to provide protection against cadmium-induced neurotoxicity.

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Evaluation of inorganic fractions of arsenic in relation to soil properties in affected areas of West Bengal, India

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Inorganic soil arsenic (As) in three soils was fractionated adopting phosphorus fractionation schemes. Among these fractions, iron-bound arsenic (Fe-As) was found highest, followed by aluminium-bound arsenic (Al-As). The freely exchangeable arsenic was relatively small compared to the arsenic held by internal surfaces of soil aggregates. The arsenic fractions exhibited positive correlation with phosphorus content presumably due to the fact that high P in soil releases more arsenic from soil adsorption sites owing to the competition for the same adsorption sites. Predominantly, negative correlation of arsenic with organic carbon confirms the fact of lowering of arsenic mobility in presence of organics in soil.

Keywords: Arsenic fractions, arsenic extractants, soil properties, resin extractable arsenic.

ARSENIC (As) is a widely occurring toxic metalloid in natural ecosystems. It is drawing global concern because of its indiscriminate contamination affecting millions in many countries including the state of West Bengal, India. The entry of arsenic into the soil–plant system occurs through either natural process of weathering of arsenic-bearing rocks and/or use of arsenic-contaminated groundwater for irrigation, or else through a host of anthropogenic activities such as mining operations, smelting of base metal ores, combustion of coal and application of arsenicals as agricultural pesticides[1–5].

Arsenic is the twentieth abundant element in the earth's crust[1]. It is distributed in soils in various forms associated with different soil constituents, namely iron, aluminium, calcium compounds, etc. forming varieties of compounds having different solubility and mobility in soil–water system[6]. Arsenic is also present in soil in adsorbed phases on clays, ferromanganese oxides and organic matter[7]. The amount of plant available arsenic is very limited compared to the total arsenic content in soil[8]. Thus, the total concentration of As in soil may be a good indicator of the degree and extent of contamination but is insufficient for evaluation of its environmental impact without considering the speciation[9]. Therefore, the

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